THE EFFECTS OF ADRENERGIC RECEPTOR ACTIVATION ON DELAYED AFTER-DEPOLARIZATION AND TRIGGERED ACTIVITY IN ISOLATED CARDIAC MYOCYTES.

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Activation of alpha-1-adrenergic receptors (AR) were shown to enhance arrhythmias induced by early phases of ischemia/reperfusion or by early after-depolarizations. However, we have observed that triggered activity induced by delayed after-depolarizations (DAD) during activation of beta adrenergic receptors by isoproterenol (1x10-9M to 5x10-8 M) in cardiac ventricular myocytes of dog and guinea pig could be reversibly abolished by methoxamine (1 to 5x10-6M) an AR agonist. This effect of methoxamine could be reversed by prazosin (1x10-6M) an AR antagonist. DAD induced by isoproterenol during intracellular stimulation at 1-3 Hz and recorded by intracellular microelectrodes (40 - 60 Mohm; 3M KCl; Axoclamp 2A) were analyzed digitally. The voltage curves of DAD were irregular with multiple peaks and their duration was 3-5 times longer than the duration of the preceding stimulated action potentials suggesting a spontaneous intracellular Ca^{2+} release which may last over 1 sec. However, if action potentials were elicited during DAD it caused an early termination of DAD. Methoxamine which abolished triggering completely did not shorten the duration of DAD but reduced their peak amplitudes and increased their coupling times by a factor of 3 to 4 suggesting that it did not reduce the spontaneous release of Ca^{2+} but it could delay the intracellular release of Ca^{2+}. Administration of prazosin during treatment with methoxamine enhanced DAD and triggering above the levels before methoxamine indicating that an activation of AR did not prevent Ca^{2+} -loading by isoproterenol. We conclude that stimulation of AR when DAD are induced by BR-activation may not reduce Ca^{2+} -loading but it may delay the intracellular release of Ca^{2+}. This delay in Ca^{2+} -release caused by activation of AR may reduce the arrhythmogenic effects of DAD because DAD which are shifted to the end of diastole have small amplitudes not sufficient for triggering and they may also be overlapped by the next normal action potential.

SODIUM CURRENT MODULATION BY AUTONOMIC TRANSMITTERS IN ISOLATED RABBIT CARDIAC MYOCYTES

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Autonomic transmitters are known to play an important role in the pathogenesis of cardiac arrhythmias, especially during settings of acute myocardial infarction or sudden cardiac arrests. We studied the effect of autonomic transmitters on voltage-dependent sodium currents in isolated rabbit ventricular myocytes using whole cell patch-clamp techniques. The sodium current was found to be potentiated by β-adrenergic stimulation with isoproterenol (1 μM). The β-adrenergic effects include: 1) a holding potential dependent increase in the sodium current, and was observed only when resting membrane potential was normal or hyperpolarized, but not at depolarized resting membrane potentials, 2) a hyperpolarizing shift in the sodium current-voltage relationship so that the amplitude of the sodium current was augmented at potentials negative to the peak, and 3) a shift of the sodium current inactivation curve in the hyperpolarizing direction. These β-adrenergic effects are reversed by propanolol (1 μM) and acetylcholine (1 μM) and are imitated by IBMX, forskolin and dibutyryl cAMP, suggesting that these effects are mediated through cAMP, possibly via phosphorylation of the α subunit of the sodium channel. Our findings suggest that impulse conduction would assume greater nonuniformity under conditions of cardiac ischemia and in the presence of β-adrenergic stimulation and may predispose the heart to development of lethal arrhythmias.

Slowly-Inactivating Sodium Current as a Source of Early Afterdepolarizations in Isolated Ventricular Myocytes

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Early afterdepolarizations (EADs) were induced in rat neonatal ventricular myocytes by exposure to anthopleurin-A (AP-A, 80-240 nM). To investigate the mechanism of EADs, whole-cell voltage clamp studies of Na currents (I_{Na}) were done, with Ca currents suppressed by low external Ca (20 uM) and Mn. In control conditions, inactivation proceeded exponentially with time constant, tau_h, averaging 1.5 ms $\pm$ .2 ms at -30 mV and peak averaged 0.7 $\pm$ 0.3 nA (n = 10). Steady state inactivation of I_{Na} was complete at -50 mV. There was little or no overlap of I_{Na} inactivation and activation processes in control. One to 3 minutes following exposure to AP-A, the rate of inactivation slowed, with the time constant, tau_h, increasing to 21 $\pm$ 5 ms at -30 mV, and peak I_{Na} increasing to 1.3 $\pm$ 0.5 nA (n = 10). Steady state inactivation was shifted in a positive direction, and complete inactivation did not occur even at + 10 mV. The long-lasting Na currents and EADs induced by AP-A resembled each other in terms of fluctuations and voltage dependence, and both were selectively antagonized by lidocaine (60 uM). Thus, slowly inactivating Na currents, possibly carried through Na channels having late kinetics, may be causally related to EADs in this model.

INTRACELLULAR PERFUSION OF CYCLIC AMP DECREASES THE SODIUM CURRENT OF GUINEA PIG VENTRICULAR MYOCYTES.

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Previous studies from our laboratory have shown that noradrenaline and isoproterenol (ISP) inhibit cardiac Na^+ current (I_{Na}) via stimulation of β-receptor, thereby suggesting an involvement of cyclic AMP (cAMP) in this phenomenon. Thus we studied the effect of cAMP on the I_{Na} of enzymatically isolated ventricular myocytes with the use of extra- and intra-cellular perfusion techniques, under whole cell voltage-clamp condition. The extracellular [Na^+] was reduced to 60 mM by replacing NaCl with sucrose and the temperature was kept at 24-26° C. Ca^{2+} and K^+ currents were blocked by appropriate channel blockers. Depolarizing clamp pulses (30 ms duration) of various amplitudes were applied at a rate of 0.2 Hz from a holding potential of -80 mV. The intracellular perfusion (30-50 μl/min) with the solution containing cAMP (200-1000 μM), for 3-6 min, decreased the peak I_{Na} from 6.3±0.8 nA to 4.3±1.0 nA (n=6, $p<0.05$) or by 32.2 %, and which was partially reversed after switching to cAMP-free intracellular perfusate. The agent shifted the relationship between the normalized I_{Na} and the membrane potential (h_∞ -Em curve) to negative direction by ≈5.0 mV (n=2). Extracellular application of dibutyryl cAMP (5 mM) had similar effects, and the effect was blocked in the presence of H8 (10 μM, MW 338) or H89 (10 μM, MW [illegible]), the most potent and specific inhibitor of A-kinase. The observations suggest that cAMP regulates cardiac I_{Na} via A-kinase mediated phosphorylation of the Na channel.